Editorial Manager(tm) for Animal Behaviour
Manuscript Draft

Manuscript Number: 8857R2

Title: The effect of habitat composition on sexual conflict in the seaweed flies, Coelopa frigida and C. pilipes

Article Type: UK Research paper

Corresponding Author: Mr Dominic Alexander Edward, MSc

Corresponding Author's Institution: University of Stilring

First Author: Dominic A Edward, MSc

Order of Authors: Dominic A Edward, MSc; Andre S Gilburn, PhD

Abstract: Considering the recent explosion of interest in sexual conflict, the effect of environmental conditions on the intensity of sexual conflict within populations has been largely ignored. Reproductive encounters within coelopids are characterised by sexual conflict in the form of intense male harassment, usually resulting in a vigorous pre-mating struggle. Here we investigated the effect of habitat composition and duration of exposure to oviposition sites on the level of sexual harassment by males and mating success in two species of European seaweed flies, Coelopa frigida and C. pilipes. The wrack beds inhabited by these two species are dominated by two genera of brown algae, Fucus and Laminaria, the relative proportions of which can vary considerably between wrack beds. Previous studies have shown that Fucus stimulates male harassment, increases copulation duration and induces females to oviposit in both species. Here we show that Laminaria stimulates a higher level of harassment in male C. frigida than Fucus. However, a similar effect was not observed in C. pilipes, with the main additional factor affecting male harassment in this species being the age of the male. Our study highlights the potential importance of environmental conditions on the intensity of sexual conflict within a population. We discuss the evolutionary significance of these observed effects in seaweed flies.

*** Cover letter**

Dominic Edward
School of Biological &
Environmental Sciences
University of Stirling
Stirling
FK9 4LA

E-mail: dominic.edward@stir.ac.uk
Tel: 07986 401223

Dr A. K. Turner
Managing Editor
Animal Behaviour Editorial Office
School of Biology
University of Nottingham
University Park
Nottingham
NG7 2RD

22nd February 2006

Dear Dr Turner,

Please find attached manuscript that we hope you would consider for publication in *Animal Behaviour*. I can confirm that the research described is original and does not overlap with other published work, nor has been submitted to other journals for publication.

Yours faithfully,

Dominic Edward

ASAB/ABS CONFIRMATION FORM

Please confirm the following:

[✓] 1. This material has not been published or submitted for publication elsewhere.

[✓] 2. All coauthors know that this manuscript has been submitted for publication.

[N/A] 3. An explanation of overlap with other articles (published or in press in journals, books or conference proceedings, or in preparation) has been included in the cover letter accompanying this manuscript.

[N/A] 4. **In the case of Forum critiques of published papers**, the author(s) of the target article has been contacted and trivial points of difference or misunderstanding resolved

[✓] 5. This research adhered to the ASAB/ABS *Guidelines for the Use of Animals in Research*, the legal requirements of the country in which the work was carried out, and all institutional guidelines. The *Guide to Ethical Information Required for* Animal Behaviour *Papers* has been consulted and requirements met.

This research was reviewed and approved by:

[ ] The University Animal Research Ethics Board **(Canada)** of
_____

(Animal Utilization Proposal No. _____).

[ ] The Institutional Animal Care and Use Committee **(U.S.A.)** of
_____

(Animal Research Protocol No. _____).

[ ] **(Outside North America)** the appropriate institutional and/or governmental authority regulating research with animals (please specify and quote any certificate or licence numbers): _____
_____.

This research was not reviewed by an institutional or governmental regulatory body because:

[ ] It is a theoretical paper or a review of the current literature.

[✓] It involved the study of species for which no such review was required.

[ ] **Other: *Please specify.*** _____
_____
_____.

Please note: ASAB's and ABS's ethical guidelines may raise issues beyond those of institutional or government ethical guidelines. At the suggestion of reviewers or the Editor, a manuscript may be reviewed by the ASAB Ethical Committee/ABS Animal Care Committee and the decision to publish may be affected by their evaluation.

The effect of habitat composition on sexual conflict in the seaweed flies, *Coelopa frigida* and *C. pilipes*

DOMINIC A. EDWARD & ANDRE S. GILBURN
School of Biological and Environmental Sciences, University of Stirling

Editors Comments

Parts of the discussion have been reworded to make the distinction between realised and potential conflict. (Lines 240-254)

Sentence beginning line 243 and ending 244 has been deleted.

On line 268-269, “to generate sexual selection” has been deleted.

In the final paragraph reference is now made to mating rate as opposed to optimal mating rate.

In the final paragraph Simmons & Bailey 1990 has been included as a reference.

Sentence beginning on line 300 and ending on line 301 has been reworded, now on lines 298-300.

Line 308 has been changed, now line 307.

The penultimate sentence, lines 312-314 has been deleted.

Typo on line 186 has been corrected.

Figures have been reduced in Excel so as to only take up a single page in the constructed PDF file.

The effect of habitat composition on sexual conflict in the seaweed flies, *Coelopa frigida* and *C. pilipes*

DOMINIC A. EDWARD & ANDRE S. GILBURN

School of Biological and Environmental Sciences, University of Stirling

Lay Summary

There are two species of seaweed fly found in Northern Europe that live in deposits of seaweed washed up on beaches at high tide. Mating in both species involves a violent struggle as females attempt to reject multiple attempts to mate made by males. We measured the number of times males attempted to mate and were successful at mating with a female under different conditions. By varying the type of seaweed that males were exposed to and how long they were exposed we found that male mating behaviour was different in the two species. Whilst the male of one species of seaweed fly was influenced by the type of seaweed, males of the other species were influenced by the length of time that they had been exposed. The variability in male mating behaviour found in seaweed flies is of interest because of the intensity of the conflict between the sexes.

The effect of habitat composition on sexual conflict in the seaweed flies, *Coelopa frigida* and *C. pilipes*

DOMINIC A. EDWARD & ANDRE S. GILBURN

School of Biological and Environmental Sciences, University of Stirling

Running Headline: **EDWARD & GILBURN: SEXUAL CONFLICT IN COELOPIDS**

Correspondence: Dominic Edward, School of Biological and Environmental Sciences, University of Stirling, Scotland, FK9 4LA, U.K. (email: dae1@stir.ac.uk).

Word Count: 3353 (excluding references and figure legends)

Considering the recent explosion of interest in sexual conflict, the effect of environmental conditions on the intensity of sexual conflict within populations has been largely ignored. Reproductive encounters within coelopids are characterised by sexual conflict in the form of intense male harassment, usually resulting in a vigorous pre-mating struggle. Here we investigated the effect of habitat composition and duration of exposure to oviposition sites on the level of sexual harassment by males and mating success in two species of European seaweed flies, *Coelopa frigida* and *C. pilipes*. The wrack beds inhabited by these two species are dominated by two genera of brown algae, *Fucus* and *Laminaria*, the relative proportions of which can vary considerably between wrack beds. Previous studies have shown that *Fucus* stimulates male harassment, increases copulation duration and induces females to oviposit in both species. Here we show that *Laminaria* stimulates a higher level of harassment in male *C. frigida* than *Fucus*. However, a similar effect was not observed in *C. pilipes*, with the main additional factor affecting male harassment in this species being the age of the male. Our study highlights the potential importance of environmental conditions on the intensity of sexual conflict within a population. We discuss the evolutionary significance of these observed effects in seaweed flies.

Following the work of Parker (1979) there has been a recent explosion of interest in sexual conflict and its importance as a major force shaping the evolution of mating systems (reviewed by Chapman et al. 2003; Arnqvist & Rowe 2005). Yet despite this upsurge in research investigating sexual conflict, the influence of environmental conditions on the intensity of conflict within populations has been largely ignored. Identifying environmental influences on sexual conflict are essential if we are to fully understand how mating systems operate and evolve (Ortigosa & Rowe 2002). A number of studies have investigated the effects of predation risk (Sih 1988; Lima & Dill 1990; Sih et al 1990) and hunger (Travers & Sih 1991) on mating behaviour but very few have actually investigated its effects directly on sexual selection and sexual conflict (Rowe 1992; Ortigosa & Rowe 2002).

Examples of mating systems characterised by high levels of male harassment and vigorous pre-mating struggles are found in many insect taxa including sepsids (Ward et al. 1992), gerrids (Rowe et al. 1994), coccinellids (Majerus 1994), carabids (Takami 2002) and ichneumonids (Teder 2005). All species of seaweed fly (Coelopidae) so far studied exhibit pre-mating struggles (Day et al. 1990; Crean & Gilburn 1998; Crean et al. 2000). Coelopids can be found inhabiting accumulations of detached seaweed deposited on the seashore after high tides or stormy weather, known as wrack beds. Seaweed is known to be important for coelopid reproduction, increasing male harassment of females and reducing survival in both sexes (Dunn et al. 2002). Seaweed also induces oviposition in females, but otherwise does not influence female reproductive behaviour, with a consistent rejection response being maintained irrespective of the presence or absence of seaweed (Dunn et al. 2002). Male harassment invariably results in a pre-mating struggle during which the female

will attempt to reject the male with a combination of shaking and kicking movements, whilst at the same time curling her abdomen downwards to prevent genital contact (Day et al. 1990). Thus, the presence of seaweed increases the frequency of pre-mating struggles within a population of coelopids.

The two species of coelopid most commonly found on the coasts of Northern Europe are *Coelopa pilipes* and *Coelopa frigida* (Phillips et al. 1995). *C. frigida* and *C. pilipes* can be found in both allopatric and, more commonly, sympatric populations. Both conspecific and heterospecific interactions between larvae may influence the success of individuals in high density populations (Phillips et al. 1995), with the mechanism of competition most likely to be purely exploitative.

The coelopid life cycle can be completed in wrack beds composed of a wide variety of different seaweeds (Dobson 1974; Phillips et al. 1995). Wrack beds around the UK are primarily composed of two different genera of brown algae, *Fucus* and *Laminaria*. Dobson (1974) reported that *C. frigida* could be bred more successfully on *Laminaria* monocultures than *C. pilipes* and also that *C. frigida* were found in greater numbers in wrack beds that predominantly consisted of *Laminaria*. *C. pilipes* females show a preference for ovipositing on *Fucus*, though it should be noted that both *C. frigida* and *C. pilipes* will lay eggs on both types of seaweed (Phillips et al. 1995).

Male harassment has previously been shown to be stimulated by the presence of *Fucus* in both *C. frigida* and *C. pilipes* (Dunn et al. 2002). Male harassment rate determines the frequency at which pre-mating struggles occur. Therefore, the level of sexual conflict within a population is affected by environmental conditions in the

form of the presence of seaweed. As previous studies have reported that *C. frigida* larvae and adults favour *Laminaria*, it might be expected that the stimulation of male harassment would also be algal specific. Additionally the duration of exposure to algae might also affect the level of stimulation of male harassment. In this study we compare the effects of different algal genera and duration of exposure to them on the harassment and copulation success rates of male *C. frigida* and *C. pilipes.* We then discuss the implications of habitat specific effects on sexual conflict and interspecific competition.

METHODS

Preparation

Laboratory populations of *C. pilipes* and *C. frigida* were established from wild larvae collected in February 2004 from the Forth Estuary. *C. frigida* were from Whitesands, East Lothian (NT712775) and *C. pilipes* from St Monans, Fife (NO521012). Virgin flies were collected and stored in 250ml flasks at 5^{o}C with cotton wool soaked in excess 5% sucrose solution. Under these conditions flies are not reproductively active. Flies were maintained for no more than 5 generations in the laboratory and used for mate trials within 7 days of eclosion. Seaweed (*Fucus serratus, Fucus vesiculosus* and *Laminaria digitata*) was collected from Whitesands, East Lothian and Cellardyke Harbour, Fife (NO577038). Seaweed was coarsely minced prior to use.

Experimental Procedure

115

116 Male flies were placed into individual plastic vials (45mm dia. X 40mm) with
117 one of four different seaweed treatments - *F. serratus*, *F. vesiculosus*, *L. digitata* or a
118 control. A small amount of minced seaweed (weighing approximately 2g) was added
119 to each vial in addition to a small ball of cotton wool soaked in excess 5% sucrose
120 solution. The control group vials contained only cotton wool soaked in sucrose
121 solution. Female flies were placed into individual clear plastic containers (30mm dia.
122 X 54mm) containing a small amount of cotton wool soaked in 5% sucrose solution.
123 Flies were initially transferred into their respective containers under light CO_2
124 anaesthesia, but for the remainder of the study no anaesthesia was used. Containers
125 were subsequently stored in constant temperature rooms at 25^{o}C prior to mate trials.
126 Female flies and control males were kept separately in a room that had no history of
127 seaweed presence. Every 24 hours additional sucrose solution was added to vials to
128 replace fluid lost by evaporation.
129

130 At intervals of 1, 2 and 3 days following preparation an equal proportion of
131 vials from each seaweed treatment group were used in mate trials. Thus a 3 x 4
132 factorial design was adopted for each fly species to determine the effect of time left on
133 the seaweed and species of seaweed upon male mating behaviour. All mate trials were
134 carried out at 25^{o}C. Males were introduced into the vials of randomly selected females
135 and observed for up to 10 minutes or until a mount was observed. Males failing to
136 mount within 10 minutes were scored as unwilling to mount. For those that did mount
137 their given female, the outcomes of pre-mating struggles were recorded as either
138 copulation (genital coupling was observed) or female rejection (if the female managed
139 to reject the male). Flies were killed by placing them in a freezer at -25^{o}C. Body size

was estimated by measuring wing length, which has been used an indicator of size in most previous studies of coelopid behaviour (e.g. Day et al. 1990; Crean & Gilburn 1998; Crean et al. 2000; Dunn et al. 2002).

Statistical Analysis

Binary logistic models of willingness to mount and copulation success rate (for those that mounted) were created separately using SPSS v12.0.1. Models were further simplified to analyse differences within each species and between treatments. Species, treatment, duration of exposure to treatment, male size, female size and the interaction terms were initially included in all models as applicable. Maximal models were selected based upon the Akaike Information Criterion calculated using R (R Development Core Team 2006), non-significant terms being excluded from models. All P-values were determined using log-likelihood chi-squares.

RESULTS

Harassment Levels

Harassment levels differed in *C. frigida* and *C. pilipes* (Chi-square test: $\chi^2_1 = 53.685$, $P < 0.001$) and were influenced by both treatment (Chi-square test: $\chi^2_3 = 13.951$, $P = 0.003$) and the number of days exposed (Chi-square test: $\chi^2_1 = 20.820$, $P < 0.001$; Fig. 1.). The influence of length of exposure to treatments differed between the species (Chi-square test: $\chi^2_1 = 13.222$, $P < 0.001$). *C. frigida* were not influenced by length of exposure (Chi-square test: $\chi^2_1 = 0.986$, $P = 0.321$) whilst *C. pilipes* males

were (Chi-square test: $\chi^2_1 = 33.255$, $P < 0.001$). Despite a non-significant interaction term, harassment by *C. frigida* males was influenced by treatment (Chi-square test: $\chi^2_3 = 9.942$, $P = 0.019$) whereas *C. pilipes* was not (Chi-square test: $\chi^2_3 = 5.644$, $P = 0.130$). The absence of a significant interaction between species and treatment may be the result of a reduced data set as fewer *C. pilipes* mounted a female than *C. frigida* (*C. pilipes*: 90 out of 286 (31%); *C. frigida*: 174 out of 281 (62%)). In particular, only 8 male *C. pilipes* mounted a female following 1 day of exposure to treatments.

Differences between *F. serratus* & *F. vesiculosus* did not contribute to the altered harassment rates in *C. frigida* (Chi-square test: $\chi^2_1 = 0.030$, $P = 0.862$) and so these treatments were combined in further analyses of male harassment. There was also no significant difference between treatment with *Fucus* seaweed and the control (Chi-square test: $\chi^2_1 = 1.476$, $P = 0.224$). Instead, the effect of different treatments for *C. frigida* was the result of differences between *Laminaria* and the control (Chi-square test: $\chi^2_1 = 9.549$, $P = 0.002$) and between *Laminaria* and *Fucus* (Chi-square test: $\chi^2_1 = 5.432$, $P = 0.020$; Fig. 1.).

Mating Success

Mating success was determined primarily by an interaction between male size and female size (Chi-square test: $\chi^2_1 = 6.981$, $P = 0.008$). This is the result of larger males being able to overcome the rejection response of smaller females more easily (Gilburn et al. 1992; Crean & Gilburn 1998). In addition, there was a difference in the success rate between the species that was dependent upon male size (Chi-square test: $\chi^2_1 = 4.890$, $P = 0.027$). In *C. frigida* successful males tended to be larger than

190 unsuccessful males whereas the difference was negligible in *C. pilipes*. Finally,
191 mating success was also determined by treatment (Chi-square test: $\chi^2_3 = 8.259$, $P =$
192 0.041).
193
194 There was a difference in mating success between different lengths of
195 exposure, however this was not significant at the 5% level when both species were
196 included (Chi-square test: $\chi^2_1 = 3.465$, $P = 0.063$). When considering *C. frigida* alone,
197 length of exposure had no effect on mating success (Chi-square test: $\chi^2_1 = 1.616$, $P =$
198 0.204) but the effect was significant for *C. pilipes* (Chi-square test: $\chi^2_1 = 4.006$, $P =$
199 0.045). Analogous with harassment levels, mating success in *C. pilipes* was not
200 affected by treatment (Chi-square test: $\chi^2_3 = 0.021$, $P = 0.999$), however male *C.*
201 *frigida* were influenced by treatment (Chi-square test: $\chi^2_3 = 11.549$, $P = 0.009$; Fig.
202 2.). As with the harassment levels, the absence of significant interaction terms in the
203 model including both species is likely the result of a bias in sample sizes as a higher
204 number of *C. frigida* copulations were recorded than *C. pilipes* (*C. pilipes*: 42 out of
205 90 (47%); *C. frigida*: 103 out of 174 (59%)). Two male *C. pilipes* copulated following
206 one day of exposure to treatment, increasing to only 14 after two days of exposure.
207
208 There was no difference in the effect of the different *Fucus* seaweeds on
209 mating success (Chi-square test: $\chi^2_1 = 2.438$, $P = 0.118$) and so these treatments were
210 again combined in further analyses. Treatment with either *Fucus* or *Laminaria*
211 seaweeds led to a significant increase in mating success of *C. frigida* (Chi-square test:
212 *Fucus* $\chi^2_1 = 5.114$, $P = 0.024$, *Laminaria* $\chi^2_1 = 9.348$, $P = 0.002$). Whilst *Laminaria*
213 resulted in a greater and more significant increase in mating success compared to the

control, there was no significant difference between the effects of *Laminaria* and *Fucus* (Chi-square test: $\chi^2_1 = 1.689$, $P = 0.194$).

DISCUSSION

Harassment of females by male *C. frigida* was stimulated by the presence of fucoid seaweeds. This finding is consistent with previous studies (Dunn et al. 2002). In the present study we exposed males to a second genus of brown algae, *Laminaria*, also commonly found in Northern European wrack beds. This genus of seaweed was found to have a greater stimulatory effect on male harassment levels than fucoid seaweeds. Thus, the relative proportion of *Laminaria* and *Fucus* within wrack beds is likely to affect the level of male harassment by *C. frigida*, and therefore determine the level of sexual conflict within each population of this species.

By contrast, seaweed species composition of a wrack bed is unlikely to affect the level of sexual conflict within *C. pilipes*. Previous studies have found increased mating activity in *C. pilipes* when exposed to algae (Dunn et al. 2002), however we show that the duration of exposure to either algae or indeed sugar solution is the primary factor determining the level of harassment within this species. Given no significant interaction between duration of exposure and treatment, it is difficult to state whether *C. pilipes* require a period of maturation and/or exposure to seaweed in order to stimulate willingness to mate. Due to low levels of harassment in *C. pilipes* relative to *C. frigida*, particularly following short periods of exposure, a much greater sample size in combination with a more defined age range of flies would be required to determine the influence of age as opposed to exposure.

Discovering that the intensity of male harassment is environmentally determined, and varies both spatially and temporally, has important implications for attempts to measure the intensity of selection occurring as a result of sexual conflict. A single measurement of the intensity of realised conflict might not be a true reflection of the level within a population. Several measurements might be required at different time points and under different environmental conditions in order to gain a clear estimate of the average level of, and variability in, the intensity of conflict occurring with a population.

Habitat variation can also influence the outcome of sexual selection. For example turbidity inhibits mate choice in cichlid fish (Seehausen et al. 1997) and availability of breeding sites determines the strength of sexual selection in the European lobster and sand goby (Forsgren et al. 1996; Debuse et al. 2003). Population density has been identified as fundamental to the evolutionary outcome of sexual selection and sexual conflict (Martin & Hosken 2003; Kokko & Rankin 2006). We show that environmental variation also impacts upon the mating system of coelopids. Whilst the proximal mechanism for this association requires further investigation, we suggest either a developmental or competitive advantage conferred to *C. frigida* by enhanced reproduction in *Laminaria* deposits.

The discovery that genus of seaweed is likely to determine the level of male harassment within a wrack bed poses the question what effect seaweed composition within a wrack bed might also have on female behaviour. *C. frigida* females might temporarily avoid areas of wrack beds with high proportions of *Laminaria* until they

are ready to mate in order to reduce harassment by males. Female *C. frigida* may also be predicted to alter their reluctance to mate when exposed to different seaweeds. As *Laminaria* is their preferred oviposition medium, the presence of this seaweed might increase resistance to male harassment if female reluctance has evolved through mate assessment (Eberhard 1996; Teder 2005). The opposite may be observed if reluctance to mate has evolved in order to avoid costs associated with mating (Rowe 1992; Rowe et al 1994; Blanckenhorn et al 2000; Dunn et al. 2002). In this case, female reluctance might be expected to be reduced in the presence of *Laminaria* as increased levels of male harassment are likely to increase the costs of resistance.

Different patterns of female reluctance are also predicted in *C. pilipes* by the mate assessment and reduced mating rate hypotheses. The mate assessment hypothesis predicts that female resistance should intensify as male harassment increases over time. By contrast, the reduced mating rate hypothesis, predicts the opposite, a reduction in female resistance over the same time period that male harassment rate increases, as a result of increased costs to resistance. Thus, the spatial and temporal effects found on male harassment levels provide us with alternative predictions for the mate assessment and reduced mating rate hypotheses. The generation of alternative predictions for these hypotheses has proved difficult and their separation has become one the most controversial areas of research within sexual conflict. This study has enabled us to generate additional alternative predictions that can be tested in future studies.

Experimental manipulation of mating rates has been fundamental to the study and understanding of processes underlying sexual conflict. This can be achieved with

289 relative ease by altering either the operational sex ratio (Arnqvist 1992; Rowe 1992;
290 Vepsalainen & Savolainen 1995; Rowe & Arnqvist 2002; Wigby & Chapman 2004)
291 or population density (Arnqvist 1992; Martin & Hosken 2003; Hardling & Kaitala
292 2005). In addition, other ecological factors such as food deprivation (Simmons &
293 Bailey 1990; Rowe 1992; Sih & Krupa 1992; Ortigosa & Rowe 2002), predation (Sih
294 & Krupa 1992; Sih 1994) and mating history (Shuker & Day 2001; Ortigosa & Rowe
295 2003) have also been found to influence the extent of sexual conflict. However, in the
296 majority of studies there is a bias towards manipulation of female mating rates, with a
297 relative inability to alter male mating behaviour (Sih & Krupa 1992; Ortigosa & Rowe
298 2002; Rowe & Arnqvist 2002). The greater reproductive investment made by females
299 in a majority of taxa would likely explain the wider variation in susceptibility to the
300 costs of mating when exposed to different environmental stresses. For example, in the
301 water striders (Gerridae) female hunger is found to influence mating frequency whilst
302 male hunger does not (Rowe 1992; Ortigosa & Rowe 2002). Similarly, a male biased
303 OSR does not affect the mating rate of male gerrids. It has been suggested that
304 optimal male mating rates are relatively high and constant among gerrids in relation to
305 females (Rowe & Arnqvist 2002). Although one study (Lauer et al. 1996) found that
306 male mating insistence, but not mating rate, was positively correlated with male
307 density. It therefore appears that interspecific variation in male mating rate is not
308 sufficient to explain behavioural covariation, which is instead most likely the result of
309 variation in female mating rates (Rowe & Arnqvist 2002). In *Coelopa* a contrasting
310 system is observed, with variation in male mating rates and mating success occurring
311 both inter- and intraspecifically. Through the manipulation of male mating behaviour,
312 coelopids provide an ideal model system in future comparative studies and population
313 crosses.

ACKNOWLEDGMENTS

We are extremely grateful to Matt Tinsley and two anonymous referees for their valuable comments on the manuscript. The research described was funded by an NERC studentship (to D.A.E.).

REFERENCES

Arnqvist, G. 1992. Pre-copulatory fighting in a water strider: inter-sexual conflict or mate assessment? *Animal Behaviour*, **43,** 559-567.

Arnqvist, G. & Rowe, L. 2005. *Sexual Conflict.* Princeton: Princeton University Press.

Blanckenhorn, **W. U., Mühlhäuser, C., Morf, C., Reusch, T. & Reuter, M.** 2000. Female choice, female reluctance to mate, and sexual selection on body size in the dung fly *Sepsis cynipsea*. *Ethology*, **106,** 577–593.

Chapman, T., Arnqvist, G., Bangham, J. & Rowe, L. 2003. Sexual conflict. *Trends in Ecology and Evolution*, **18,** 41-47.

Crean, C. S. & Gilburn, A. S. 1998. Sexual selection as a side-effect of sexual conflict in the seaweed fly, *Coelopa ursina* (Diptera: Coelopidae). *Animal Behaviour*, **56,** 1405–1410.

Crean, C. S., Dunn, D. W., Day, T. H. & Gilburn, A. S. 2000. Female mate choice for large male size in several species of seaweed flies (Diptera: Coelopidae). *Animal Behaviour*, **58,** 121-126

Day, T. H., Foster, S. P. & Engelhard, G. 1990. Mating behavior in seaweed flies (*Coelopa frigida*). *Journal of Insect Behavior*, **3**, 105-120.

Debuse, V. J., Addison, J. T. & Reynolds, J. D. 2003. Effects of breeding site density on competition and sexual selection in the European lobster. *Behavioral Ecology,* **14,** 396–402.

Dobson, T. 1974. Mechanisms controlling species composition in natural populations of the seaweed *Coelopa*. *Journal of Natural History*, **8,** 653-673.

Dunn, D. W., Crean, C. S. & Gilburn, A. S. 2002. The effects of exposure to seaweed on willingness to mate, oviposition and longevity in seaweed flies (Diptera: Coelopidae). *Ecological Entomology*, **27**, 554-564.

Eberhard, W. G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton: Princeton University Press.

Forsgren, E., Kvarnemo, C. & Lindström, K. 1996. Mode of sexual selection determined by resource abundance in two sand goby populations. *Evolution,* **50,** 646–654.

Gilburn, A. S., Foster, S. P. & Day, T. H. 1992. Female mating preference for large size in Coelopa frigida (seaweed fly), *Heredity,* **69,** 209-216.

Hardling, R. & Kaitala, A. 2005. The evolution of repeated mating under sexual conflict. *Journal of Evolutionary Biology,* **18,** 106-115.

Kokko, H. & Rankin, D. J. 2006. Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **361,** 319-334.

Lauer, M. J., Sih, A. & Krupa, J. J. 1996. Male density, female density and inter-sexual conflict in a stream-dwelling insect. *Animal Behaviour*, **52,** 929–939.

Lima, S. L. & Dill, L. M. 1990. Behavioural decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, **68,** 619–640.

Majerus, M. E. N. 1994. *Ladybirds*. London: Harper Collins.

Martin, O. Y. & Hosken, D. J. 2003. The evolution of reproductive isolation through sexual conflict. *Nature,* **423,** 979-981.

Ortigosa, A. & Rowe, L. 2002. The effect of hunger on mating behaviour and sexual selection for male body size in *Gerris buenoi. Animal Behaviour*, **64,** 369–375.

Ortigosa, A. & Rowe, L. 2003. The role of mating history and male size in determining mating behaviours and sexual conflict in a water strider. *Animal Behaviour,* 65, 851-858.

Parker, G. A. 1979. Sexual selection and sexual conflict. In: *Sexual Selection and Reproductive Competition in Insects* (Ed. by M. S. Blum & N. A. Blum), pp. 123-166. New York: Academic Press.

Phillips, D. S., Leggett, M., Wilcockson, R., Day, T. H. & Arthur, W. 1995. Coexistence of competing species of seaweed flies: the role of temperature. *Ecological Entomology*, **20**, 65-74.

R Development Core Team 2006. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Rowe, L. 1992. Convenience polyandry in a water strider: foraging conflicts and female control of copulation frequency and guarding duration. *Animal Behaviour*, **44,** 189–202.

Rowe, L. & Arnqvist, G. 2002. Sexually antagonistic coevolution in a mating system: Combining experimental and comparative approaches to address evolutionary processes. *Evolution,* **56,** 754-767.

Rowe, L., Arnqvist, G., Sih, A. & Krupa, J. J. 1994. Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. *Trends in Ecology and Evolution*, **9,** 289–293.

Seehausen, O., van Alphen, J. J. M. & Witte, F. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science,* **277,** 1808-1811.

Shuker, D. M & Day, T. H. 2001. The repeatability of a sexual conflict over mating. *Animal Behaviour,* **61,** 755-762.

Sih, A. 1988. The effects of predators on habitat use, activity and mating behaviour of a semi-aquatic bug. *Animal Behaviour*, **36,** 1846–1848.

Sih, A. 1994. Predation risk and the evolutionary ecology of reproductive behavior. *Journal of Fish Biology,* **45,** 111-130. Suppl. A.

Sih, A. & Krupa, J. J. 1992. Predation risk, food deprivation and non-random mating by size in the stream water strider, *Aquarius remigis*. *Behavioural Ecology & Sociobiology,* **31,** 51-56.

Sih, A., Krupa, J. J. & Travers, S. E. 1990. An experimental study on the effects of predation risk and feeding regime on the mating behaviour of the water strider. *American Naturalist*, **135,** 284–290.

Simmons, L. W. & Bailey W. J. 1990. Resource influenced sex roles of Zaprochiline Tettigoniids (Orthoptera: Tettigoniidae). *Evolution,* **44,** 1853-1868.

Takami, Y. 2002. Mating behaviour, insemination and sperm transfer in the ground beetle *Carabus insulicola*. *Zoological Science,* **19**, 1067-1073.

Teder, T. 2005. Male-biased size dimorphism in ichneumonine wasps (Hymenoptera: Ichneumonidae) – the role of sexual selection for large male size. *Ecological Entomology*, **30,** 342-349.

Travers, S. E. & Sih, A. 1991. The influence of starvation and predators on the mating behaviour of a semiaquatic insect. *Ecology*, **72,** 2123–2136.

Vepsalainen, K. & Savolainen, R. 1995. Operational sex ratios and mating conflict between the sexes in the water strider *Gerris lacustris*. *American Naturalist,* **146,** 869-880.

Ward, P. I., Hemmi, J. & Röösli, T. 1992. Sexual conflict in the dung fly *Sepsis cynipsea*. *Functional Ecology*, **6**, 649-653.

Wigby, S. & Chapman, T. 2004. Female resistance to male harm evolves in response to manipulation of sexual conflict. *Evolution,* **58,** 1028-1037.

The effect of habitat composition on sexual conflict in the seaweed flies, *Coelopa frigida* and *C. pilipes*

DOMINIC A. EDWARD & ANDRE S. GILBURN

School of Biological and Environmental Sciences, University of Stirling

Running Headline: **EDWARD & GILBURN: SEXUAL CONFLICT IN COELOPIDS**

Correspondence: Dominic Edward, School of Biological and Environmental Sciences, University of Stirling, Scotland, FK9 4LA, U.K. (email: dae1@stir.ac.uk).

Word Count: 3353 (excluding references and figure legends)

Considering the recent explosion of interest in sexual conflict, the effect of environmental conditions on the intensity of sexual conflict within populations has been largely ignored. Reproductive encounters within coelopids are characterised by sexual conflict in the form of intense male harassment, usually resulting in a vigorous pre-mating struggle. Here we investigated the effect of habitat composition and duration of exposure to oviposition sites on the level of sexual harassment by males and mating success in two species of European seaweed flies, *Coelopa frigida* and *C. pilipes*. The wrack beds inhabited by these two species are dominated by two genera of brown algae, *Fucus* and *Laminaria*, the relative proportions of which can vary considerably between wrack beds. Previous studies have shown that *Fucus* stimulates male harassment, increases copulation duration and induces females to oviposit in both species. Here we show that *Laminaria* stimulates a higher level of harassment in male *C. frigida* than *Fucus*. However, a similar effect was not observed in *C. pilipes*, with the main additional factor affecting male harassment in this species being the age of the male. Our study highlights the potential importance of environmental conditions on the intensity of sexual conflict within a population. We discuss the evolutionary significance of these observed effects in seaweed flies.

Following the work of Parker (1979) there has been a recent explosion of interest in sexual conflict and its importance as a major force shaping the evolution of mating systems (reviewed by Chapman et al. 2003; Arnqvist & Rowe 2005). Yet despite this upsurge in research investigating sexual conflict, the influence of environmental conditions on the intensity of conflict within populations has been largely ignored. Identifying environmental influences on sexual conflict are essential if we are to fully understand how mating systems operate and evolve (Ortigosa & Rowe 2002). A number of studies have investigated the effects of predation risk (Sih 1988; Lima & Dill 1990; Sih et al 1990) and hunger (Travers & Sih 1991) on mating behaviour but very few have actually investigated its effects directly on sexual selection and sexual conflict (Rowe 1992; Ortigosa & Rowe 2002).

Examples of mating systems characterised by high levels of male harassment and vigorous pre-mating struggles are found in many insect taxa including sepsids (Ward et al. 1992), gerrids (Rowe et al. 1994), coccinellids (Majerus 1994), carabids (Takami 2002) and ichneumonids (Teder 2005). All species of seaweed fly (Coelopidae) so far studied exhibit pre-mating struggles (Day et al. 1990; Crean & Gilburn 1998; Crean et al. 2000). Coelopids can be found inhabiting accumulations of detached seaweed deposited on the seashore after high tides or stormy weather, known as wrack beds. Seaweed is known to be important for coelopid reproduction, increasing male harassment of females and reducing survival in both sexes (Dunn et al. 2002). Seaweed also induces oviposition in females, but otherwise does not influence female reproductive behaviour, with a consistent rejection response being maintained irrespective of the presence or absence of seaweed (Dunn et al. 2002). Male harassment invariably results in a pre-mating struggle during which the female

will attempt to reject the male with a combination of shaking and kicking movements, whilst at the same time curling her abdomen downwards to prevent genital contact (Day et al. 1990). Thus, the presence of seaweed increases the frequency of pre-mating struggles within a population of coelopids.

The two species of coelopid most commonly found on the coasts of Northern Europe are *Coelopa pilipes* and *Coelopa frigida* (Phillips et al. 1995). *C. frigida* and *C. pilipes* can be found in both allopatric and, more commonly, sympatric populations. Both conspecific and heterospecific interactions between larvae may influence the success of individuals in high density populations (Phillips et al. 1995), with the mechanism of competition most likely to be purely exploitative.

The coelopid life cycle can be completed in wrack beds composed of a wide variety of different seaweeds (Dobson 1974; Phillips et al. 1995). Wrack beds around the UK are primarily composed of two different genera of brown algae, *Fucus* and *Laminaria*. Dobson (1974) reported that *C. frigida* could be bred more successfully on *Laminaria* monocultures than *C. pilipes* and also that *C. frigida* were found in greater numbers in wrack beds that predominantly consisted of *Laminaria*. *C. pilipes* females show a preference for ovipositing on *Fucus*, though it should be noted that both *C. frigida* and *C. pilipes* will lay eggs on both types of seaweed (Phillips et al. 1995).

Male harassment has previously been shown to be stimulated by the presence of *Fucus* in both *C. frigida* and *C. pilipes* (Dunn et al. 2002). Male harassment rate determines the frequency at which pre-mating struggles occur. Therefore, the level of sexual conflict within a population is affected by environmental conditions in the

form of the presence of seaweed. As previous studies have reported that *C. frigida* larvae and adults favour *Laminaria*, it might be expected that the stimulation of male harassment would also be algal specific. Additionally the duration of exposure to algae might also affect the level of stimulation of male harassment. In this study we compare the effects of different algal genera and duration of exposure to them on the harassment and copulation success rates of male *C. frigida* and *C. pilipes.* We then discuss the implications of habitat specific effects on sexual conflict and interspecific competition.

METHODS

Preparation

Laboratory populations of *C. pilipes* and *C. frigida* were established from wild larvae collected in February 2004 from the Forth Estuary. *C. frigida* were from Whitesands, East Lothian (NT712775) and *C. pilipes* from St Monans, Fife (NO521012). Virgin flies were collected and stored in 250ml flasks at 5^{o}C with cotton wool soaked in excess 5% sucrose solution. Under these conditions flies are not reproductively active. Flies were maintained for no more than 5 generations in the laboratory and used for mate trials within 7 days of eclosion. Seaweed (*Fucus serratus, Fucus vesiculosus* and *Laminaria digitata*) was collected from Whitesands, East Lothian and Cellardyke Harbour, Fife (NO577038). Seaweed was coarsely minced prior to use.

Experimental Procedure

115

116 Male flies were placed into individual plastic vials (45mm dia. X 40mm) with
117 one of four different seaweed treatments - *F. serratus*, *F. vesiculosus*, *L. digitata* or a
118 control. A small amount of minced seaweed (weighing approximately 2g) was added
119 to each vial in addition to a small ball of cotton wool soaked in excess 5% sucrose
120 solution. The control group vials contained only cotton wool soaked in sucrose
121 solution. Female flies were placed into individual clear plastic containers (30mm dia.
122 X 54mm) containing a small amount of cotton wool soaked in 5% sucrose solution.
123 Flies were initially transferred into their respective containers under light CO_2
124 anaesthesia, but for the remainder of the study no anaesthesia was used. Containers
125 were subsequently stored in constant temperature rooms at 25^{o}C prior to mate trials.
126 Female flies and control males were kept separately in a room that had no history of
127 seaweed presence. Every 24 hours additional sucrose solution was added to vials to
128 replace fluid lost by evaporation.
129

130 At intervals of 1, 2 and 3 days following preparation an equal proportion of
131 vials from each seaweed treatment group were used in mate trials. Thus a 3 x 4
132 factorial design was adopted for each fly species to determine the effect of time left on
133 the seaweed and species of seaweed upon male mating behaviour. All mate trials were
134 carried out at 25^{o}C. Males were introduced into the vials of randomly selected females
135 and observed for up to 10 minutes or until a mount was observed. Males failing to
136 mount within 10 minutes were scored as unwilling to mount. For those that did mount
137 their given female, the outcomes of pre-mating struggles were recorded as either
138 copulation (genital coupling was observed) or female rejection (if the female managed
139 to reject the male). Flies were killed by placing them in a freezer at -25^{o}C. Body size

was estimated by measuring wing length, which has been used an indicator of size in most previous studies of coelopid behaviour (e.g. Day et al. 1990; Crean & Gilburn 1998; Crean et al. 2000; Dunn et al. 2002).

Statistical Analysis

Binary logistic models of willingness to mount and copulation success rate (for those that mounted) were created separately using SPSS v12.0.1. Models were further simplified to analyse differences within each species and between treatments. Species, treatment, duration of exposure to treatment, male size, female size and the interaction terms were initially included in all models as applicable. Maximal models were selected based upon the Akaike Information Criterion calculated using R (R Development Core Team 2006), non-significant terms being excluded from models. All P-values were determined using log-likelihood chi-squares.

RESULTS

Harassment Levels

Harassment levels differed in *C. frigida* and *C. pilipes* (Chi-square test: $\chi^2_1 = 53.685$, $P < 0.001$) and were influenced by both treatment (Chi-square test: $\chi^2_3 = 13.951$, $P = 0.003$) and the number of days exposed (Chi-square test: $\chi^2_1 = 20.820$, $P < 0.001$; Fig. 1.). The influence of length of exposure to treatments differed between the species (Chi-square test: $\chi^2_1 = 13.222$, $P < 0.001$). *C. frigida* were not influenced by length of exposure (Chi-square test: $\chi^2_1 = 0.986$, $P = 0.321$) whilst *C. pilipes* males

were (Chi-square test: $\chi^2_1 = 33.255$, $P < 0.001$). Despite a non-significant interaction term, harassment by *C. frigida* males was influenced by treatment (Chi-square test: $\chi^2_3 = 9.942$, $P = 0.019$) whereas *C. pilipes* was not (Chi-square test: $\chi^2_3 = 5.644$, $P = 0.130$). The absence of a significant interaction between species and treatment may be the result of a reduced data set as fewer *C. pilipes* mounted a female than *C. frigida* (*C. pilipes*: 90 out of 286 (31%); *C. frigida*: 174 out of 281 (62%)). In particular, only 8 male *C. pilipes* mounted a female following 1 day of exposure to treatments.

Differences between *F. serratus* & *F. vesiculosus* did not contribute to the altered harassment rates in *C. frigida* (Chi-square test: $\chi^2_1 = 0.030$, $P = 0.862$) and so these treatments were combined in further analyses of male harassment. There was also no significant difference between treatment with *Fucus* seaweed and the control (Chi-square test: $\chi^2_1 = 1.476$, $P = 0.224$). Instead, the effect of different treatments for *C. frigida* was the result of differences between *Laminaria* and the control (Chi-square test: $\chi^2_1 = 9.549$, $P = 0.002$) and between *Laminaria* and *Fucus* (Chi-square test: $\chi^2_1 = 5.432$, $P = 0.020$; Fig. 1.).

Mating Success

Mating success was determined primarily by an interaction between male size and female size (Chi-square test: $\chi^2_1 = 6.981$, $P = 0.008$). This is the result of larger males being able to overcome the rejection response of smaller females more easily (Gilburn et al. 1992; Crean & Gilburn 1998). In addition, there was a difference in the success rate between the species that was dependent upon male size (Chi-square test: $\chi^2_1 = 4.890$, $P = 0.027$). In *C. frigida* successful males tended to be larger than

190 unsuccessful males whereas the difference was negligible in *C. pilipes*. Finally,
191 mating success was also determined by treatment (Chi-square test: $\chi^2_3 = 8.259$, $P =$
192 0.041).
193
194 There was a difference in mating success between different lengths of
195 exposure, however this was not significant at the 5% level when both species were
196 included (Chi-square test: $\chi^2_1 = 3.465$, $P = 0.063$). When considering *C. frigida* alone,
197 length of exposure had no effect on mating success (Chi-square test: $\chi^2_1 = 1.616$, $P =$
198 0.204) but the effect was significant for *C. pilipes* (Chi-square test: $\chi^2_1 = 4.006$, $P =$
199 0.045). Analogous with harassment levels, mating success in *C. pilipes* was not
200 affected by treatment (Chi-square test: $\chi^2_3 = 0.021$, $P = 0.999$), however male *C.*
201 *frigida* were influenced by treatment (Chi-square test: $\chi^2_3 = 11.549$, $P = 0.009$; Fig.
202 2.). As with the harassment levels, the absence of significant interaction terms in the
203 model including both species is likely the result of a bias in sample sizes as a higher
204 number of *C. frigida* copulations were recorded than *C. pilipes* (*C. pilipes*: 42 out of
205 90 (47%); *C. frigida*: 103 out of 174 (59%)). Two male *C. pilipes* copulated following
206 one day of exposure to treatment, increasing to only 14 after two days of exposure.
207
208 There was no difference in the effect of the different *Fucus* seaweeds on
209 mating success (Chi-square test: $\chi^2_1 = 2.438$, $P = 0.118$) and so these treatments were
210 again combined in further analyses. Treatment with either *Fucus* or *Laminaria*
211 seaweeds led to a significant increase in mating success of *C. frigida* (Chi-square test:
212 *Fucus* $\chi^2_1 = 5.114$, $P = 0.024$, *Laminaria* $\chi^2_1 = 9.348$, $P = 0.002$). Whilst *Laminaria*
213 resulted in a greater and more significant increase in mating success compared to the

control, there was no significant difference between the effects of *Laminaria* and *Fucus* (Chi-square test: $\chi^2_1 = 1.689$, $P = 0.194$).

DISCUSSION

Harassment of females by male *C. frigida* was stimulated by the presence of fucoid seaweeds. This finding is consistent with previous studies (Dunn et al. 2002). In the present study we exposed males to a second genus of brown algae, *Laminaria*, also commonly found in Northern European wrack beds. This genus of seaweed was found to have a greater stimulatory effect on male harassment levels than fucoid seaweeds. Thus, the relative proportion of *Laminaria* and *Fucus* within wrack beds is likely to affect the level of male harassment by *C. frigida*, and therefore determine the level of sexual conflict within each population of this species.

By contrast, seaweed species composition of a wrack bed is unlikely to affect the level of sexual conflict within *C. pilipes*. Previous studies have found increased mating activity in *C. pilipes* when exposed to algae (Dunn et al. 2002), however we show that the duration of exposure to either algae or indeed sugar solution is the primary factor determining the level of harassment within this species. Given no significant interaction between duration of exposure and treatment, it is difficult to state whether *C. pilipes* require a period of maturation and/or exposure to seaweed in order to stimulate willingness to mate. Due to low levels of harassment in *C. pilipes* relative to *C. frigida*, particularly following short periods of exposure, a much greater sample size in combination with a more defined age range of flies would be required to determine the influence of age as opposed to exposure.

Discovering that the intensity of male harassment is environmentally determined, and varies both spatially and temporally, has important implications for attempts to measure the intensity of selection occurring as a result of sexual conflict. A single measurement of the intensity of realised conflict might not be a true reflection of the level within a population. Several measurements might be required at different time points and under different environmental conditions in order to gain a clear estimate of the average level of, and variability in, the intensity of conflict occurring with a population.

Habitat variation can also influence the outcome of sexual selection. For example turbidity inhibits mate choice in cichlid fish (Seehausen et al. 1997) and availability of breeding sites determines the strength of sexual selection in the European lobster and sand goby (Forsgren et al. 1996; Debuse et al. 2003). Population density has been identified as fundamental to the evolutionary outcome of sexual selection and sexual conflict (Martin & Hosken 2003; Kokko & Rankin 2006). We show that environmental variation also impacts upon the mating system of coelopids. Whilst the proximal mechanism for this association requires further investigation, we suggest either a developmental or competitive advantage conferred to *C. frigida* by enhanced reproduction in *Laminaria* deposits.

The discovery that genus of seaweed is likely to determine the level of male harassment within a wrack bed poses the question what effect seaweed composition within a wrack bed might also have on female behaviour. *C. frigida* females might temporarily avoid areas of wrack beds with high proportions of *Laminaria* until they

are ready to mate in order to reduce harassment by males. Female *C. frigida* may also be predicted to alter their reluctance to mate when exposed to different seaweeds. As *Laminaria* is their preferred oviposition medium, the presence of this seaweed might increase resistance to male harassment if female reluctance has evolved through mate assessment (Eberhard 1996; Teder 2005). The opposite may be observed if reluctance to mate has evolved in order to avoid costs associated with mating (Rowe 1992; Rowe et al 1994; Blanckenhorn et al 2000; Dunn et al. 2002). In this case, female reluctance might be expected to be reduced in the presence of *Laminaria* as increased levels of male harassment are likely to increase the costs of resistance.

Different patterns of female reluctance are also predicted in *C. pilipes* by the mate assessment and reduced mating rate hypotheses. The mate assessment hypothesis predicts that female resistance should intensify as male harassment increases over time. By contrast, the reduced mating rate hypothesis, predicts the opposite, a reduction in female resistance over the same time period that male harassment rate increases, as a result of increased costs to resistance. Thus, the spatial and temporal effects found on male harassment levels provide us with alternative predictions for the mate assessment and reduced mating rate hypotheses. The generation of alternative predictions for these hypotheses has proved difficult and their separation has become one the most controversial areas of research within sexual conflict. This study has enabled us to generate additional alternative predictions that can be tested in future studies.

Experimental manipulation of mating rates has been fundamental to the study and understanding of processes underlying sexual conflict. This can be achieved with

289 relative ease by altering either the operational sex ratio (Arnqvist 1992; Rowe 1992;
290 Vepsalainen & Savolainen 1995; Rowe & Arnqvist 2002; Wigby & Chapman 2004)
291 or population density (Arnqvist 1992; Martin & Hosken 2003; Hardling & Kaitala
292 2005). In addition, other ecological factors such as food deprivation (Simmons &
293 Bailey 1990; Rowe 1992; Sih & Krupa 1992; Ortigosa & Rowe 2002), predation (Sih
294 & Krupa 1992; Sih 1994) and mating history (Shuker & Day 2001; Ortigosa & Rowe
295 2003) have also been found to influence the extent of sexual conflict. However, in the
296 majority of studies there is a bias towards manipulation of female mating rates, with a
297 relative inability to alter male mating behaviour (Sih & Krupa 1992; Ortigosa & Rowe
298 2002; Rowe & Arnqvist 2002). The greater reproductive investment made by females
299 in a majority of taxa would likely explain the wider variation in susceptibility to the
300 costs of mating when exposed to different environmental stresses. For example, in the
301 water striders (Gerridae) female hunger is found to influence mating frequency whilst
302 male hunger does not (Rowe 1992; Ortigosa & Rowe 2002). Similarly, a male biased
303 OSR does not affect the mating rate of male gerrids. It has been suggested that
304 optimal male mating rates are relatively high and constant among gerrids in relation to
305 females (Rowe & Arnqvist 2002). Although one study (Lauer et al. 1996) found that
306 male mating insistence, but not mating rate, was positively correlated with male
307 density. It therefore appears that interspecific variation in male mating rate is not
308 sufficient to explain behavioural covariation, which is instead most likely the result of
309 variation in female mating rates (Rowe & Arnqvist 2002). In *Coelopa* a contrasting
310 system is observed, with variation in male mating rates and mating success occurring
311 both inter- and intraspecifically. Through the manipulation of male mating behaviour,
312 coelopids provide an ideal model system in future comparative studies and population
313 crosses.

ACKNOWLEDGMENTS

We are extremely grateful to Matt Tinsley and two anonymous referees for their valuable comments on the manuscript. The research described was funded by an NERC studentship (to D.A.E.).

REFERENCES

Arnqvist, G. 1992. Pre-copulatory fighting in a water strider: inter-sexual conflict or mate assessment? *Animal Behaviour*, **43,** 559-567.

Arnqvist, G. & Rowe, L. 2005. *Sexual Conflict.* Princeton: Princeton University Press.

Blanckenhorn, **W. U., Mühlhäuser, C., Morf, C., Reusch, T. & Reuter, M.** 2000. Female choice, female reluctance to mate, and sexual selection on body size in the dung fly *Sepsis cynipsea*. *Ethology*, **106,** 577–593.

Chapman, T., Arnqvist, G., Bangham, J. & Rowe, L. 2003. Sexual conflict. *Trends in Ecology and Evolution*, **18,** 41-47.

Crean, C. S. & Gilburn, A. S. 1998. Sexual selection as a side-effect of sexual conflict in the seaweed fly, *Coelopa ursina* (Diptera: Coelopidae). *Animal Behaviour*, **56,** 1405–1410.

Crean, C. S., Dunn, D. W., Day, T. H. & Gilburn, A. S. 2000. Female mate choice for large male size in several species of seaweed flies (Diptera: Coelopidae). *Animal Behaviour*, **58,** 121-126

Day, T. H., Foster, S. P. & Engelhard, G. 1990. Mating behavior in seaweed flies (*Coelopa frigida*). *Journal of Insect Behavior*, **3**, 105-120.

Debuse, V. J., Addison, J. T. & Reynolds, J. D. 2003. Effects of breeding site density on competition and sexual selection in the European lobster. *Behavioral Ecology,* **14,** 396–402.

Dobson, T. 1974. Mechanisms controlling species composition in natural populations of the seaweed *Coelopa*. *Journal of Natural History*, **8,** 653-673.

Dunn, D. W., Crean, C. S. & Gilburn, A. S. 2002. The effects of exposure to seaweed on willingness to mate, oviposition and longevity in seaweed flies (Diptera: Coelopidae). *Ecological Entomology*, **27**, 554-564.

Eberhard, W. G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton: Princeton University Press.

Forsgren, E., Kvarnemo, C. & Lindström, K. 1996. Mode of sexual selection determined by resource abundance in two sand goby populations. *Evolution,* **50,** 646–654.

Gilburn, A. S., Foster, S. P. & Day, T. H. 1992. Female mating preference for large size in Coelopa frigida (seaweed fly), *Heredity,* **69,** 209-216.

Hardling, R. & Kaitala, A. 2005. The evolution of repeated mating under sexual conflict. *Journal of Evolutionary Biology,* **18,** 106-115.

Kokko, H. & Rankin, D. J. 2006. Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **361,** 319-334.

Lauer, M. J., Sih, A. & Krupa, J. J. 1996. Male density, female density and inter-sexual conflict in a stream-dwelling insect. *Animal Behaviour*, **52,** 929–939.

Lima, S. L. & Dill, L. M. 1990. Behavioural decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, **68,** 619–640.

Majerus, M. E. N. 1994. *Ladybirds*. London: Harper Collins.

Martin, O. Y. & Hosken, D. J. 2003. The evolution of reproductive isolation through sexual conflict. *Nature,* **423,** 979-981.

Ortigosa, A. & Rowe, L. 2002. The effect of hunger on mating behaviour and sexual selection for male body size in *Gerris buenoi. Animal Behaviour*, **64,** 369–375.

Ortigosa, A. & Rowe, L. 2003. The role of mating history and male size in determining mating behaviours and sexual conflict in a water strider. *Animal Behaviour,* 65, 851-858.

Parker, G. A. 1979. Sexual selection and sexual conflict. In: *Sexual Selection and Reproductive Competition in Insects* (Ed. by M. S. Blum & N. A. Blum), pp. 123-166. New York: Academic Press.

Phillips, D. S., Leggett, M., Wilcockson, R., Day, T. H. & Arthur, W. 1995. Coexistence of competing species of seaweed flies: the role of temperature. *Ecological Entomology*, **20**, 65-74.

R Development Core Team 2006. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Rowe, L. 1992. Convenience polyandry in a water strider: foraging conflicts and female control of copulation frequency and guarding duration. *Animal Behaviour*, **44,** 189–202.

Rowe, L. & Arnqvist, G. 2002. Sexually antagonistic coevolution in a mating system: Combining experimental and comparative approaches to address evolutionary processes. *Evolution,* **56,** 754-767.

Rowe, L., Arnqvist, G., Sih, A. & Krupa, J. J. 1994. Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. *Trends in Ecology and Evolution*, **9,** 289–293.

Seehausen, O., van Alphen, J. J. M. & Witte, F. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science,* **277,** 1808-1811.

Shuker, D. M & Day, T. H. 2001. The repeatability of a sexual conflict over mating. *Animal Behaviour,* **61,** 755-762.

Sih, A. 1988. The effects of predators on habitat use, activity and mating behaviour of a semi-aquatic bug. *Animal Behaviour*, **36,** 1846–1848.

Sih, A. 1994. Predation risk and the evolutionary ecology of reproductive behavior. *Journal of Fish Biology,* **45,** 111-130. Suppl. A.

Sih, A. & Krupa, J. J. 1992. Predation risk, food deprivation and non-random mating by size in the stream water strider, *Aquarius remigis*. *Behavioural Ecology & Sociobiology,* **31,** 51-56.

Sih, A., Krupa, J. J. & Travers, S. E. 1990. An experimental study on the effects of predation risk and feeding regime on the mating behaviour of the water strider. *American Naturalist*, **135,** 284–290.

Simmons, L. W. & Bailey W. J. 1990. Resource influenced sex roles of Zaprochiline Tettigoniids (Orthoptera: Tettigoniidae). *Evolution,* **44,** 1853-1868.

Takami, Y. 2002. Mating behaviour, insemination and sperm transfer in the ground beetle *Carabus insulicola*. *Zoological Science,* **19**, 1067-1073.

Teder, T. 2005. Male-biased size dimorphism in ichneumonine wasps (Hymenoptera: Ichneumonidae) – the role of sexual selection for large male size. *Ecological Entomology*, **30,** 342-349.

Travers, S. E. & Sih, A. 1991. The influence of starvation and predators on the mating behaviour of a semiaquatic insect. *Ecology*, **72,** 2123–2136.

Vepsalainen, K. & Savolainen, R. 1995. Operational sex ratios and mating conflict between the sexes in the water strider *Gerris lacustris*. *American Naturalist,* **146,** 869-880.

Ward, P. I., Hemmi, J. & Röösli, T. 1992. Sexual conflict in the dung fly *Sepsis cynipsea*. *Functional Ecology*, **6**, 649-653.

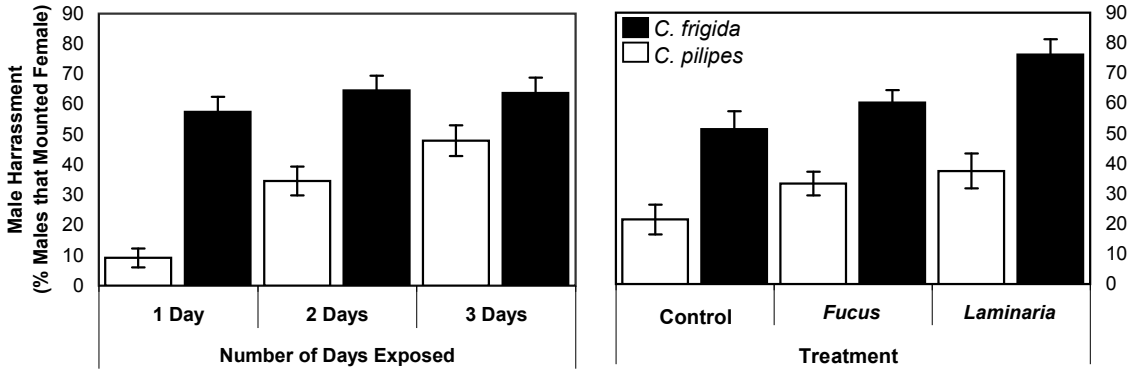
Wigby, S. & Chapman, T. 2004. Female resistance to male harm evolves in response to manipulation of sexual conflict. *Evolution,* **58,** 1028-1037.

Figure Captions

Figure 1. The effect of duration of exposure and different treatments on harassment levels in *C. frigida* & *C. pilipes*.

Figure 2. The effect of duration of exposure and different treatments on mating success of male *C. frigida* & *C. pilipes*.

Figure 1


Male Harrassment
(% Males that Mounted Female)
90
80
70
60
50
40
30
20
10
0
1 Day
2 Days
3 Days
Number of Days Exposed
C. frigida
C. pilipes
Control
Fucus
Laminaria
Treatment

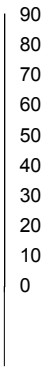
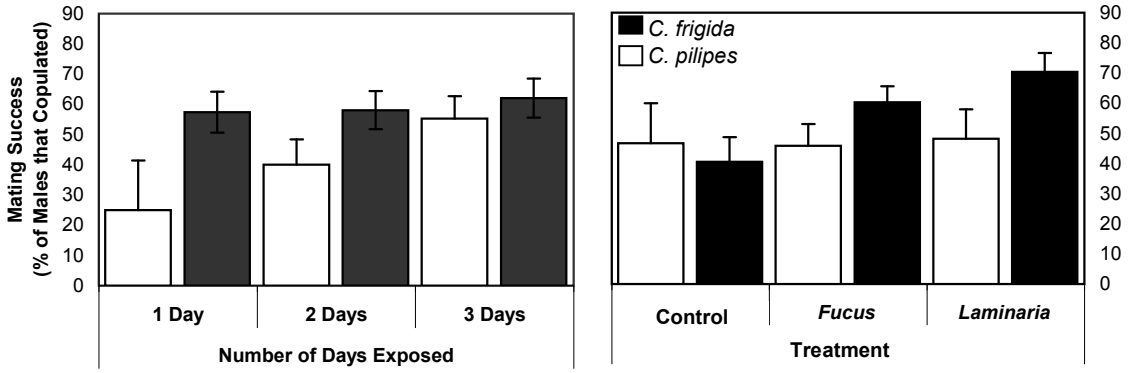
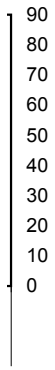

90
80
70
60
50
40
30
20
10
0

Figure 2


Mating Success
(% of Males that Copulated)
90
80
70
60
50
40
30
20
10
0
1 Day
2 Days
3 Days
Number of Days Exposed
C. frigida
C. pilipes
Control
Fucus
Laminaria
Treatment


90
80
70
60
50
40
30
20
10
0